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I. APLYSIA; II. MYZOSTOMA; III. CHAETOPTERUS

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RESEARCH ON THE MORPHOGENESIS AND DETERMINISM OF UNEQUAL SEGMENTATIONS IN SPIRALIA
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J.Pasteels[1)]

ABSTRACT. The morphogenesis and determinism of unequal segmentations in Spiralia (Aplysia, Myzostoma, and Chaetopterus) is described in detail, with discussion of various theories by different authors. Equalization of unequal segmentation, produced by ultraviolet irradiation, centrifugation, freezing, and narcotization, applied at the moment of division, during maturation, or even to the virgin ovum, resulted in the development of duplicitas. The mechanism of unequal segmentations was found to be unrelated with either unequal division of the centrosome, asymmetric special structures of the achromatic spindle, or intervention of dyschronism. The hypothesis for this mechanism postulates a differing distribution and condensation of substances, analogous to that of the polar cytoplasm and offering a certain resistance to cytodieretic processes. These substances would be germinal localizations, thus explaining the link between unequal segmentation and morphogenesis.

Introduction

The term "Spiralia" coined by Schleip (1929), designates a group of invertebrates, differing from the zoological viewpoint (Nemertinea, Annelida, Rotifera, Sipunculoideae, and Gephyrea, Hirudinea, Mollusca except for Cephalopoda) but representing an incontestible entity from the embryological viewpoint.

In these, the segmentation is strictly determined and, in addition, subject to highly distinct characteristics: alternating "spiral" rotation of the micromeres at the instant of their ejection, unequal divisions well-defined for each case and not reducible to general rules having to do with the content of vitellus.

Whereas the first of these characteristics - spiral division - is specifically difficult to subject to experimentation*, this is not the case for unequal division.

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* So far as we know, the literature contains only a single case of experimental modification of the spiral division: Of the daughter nuclei of a Crepidula ovum treated by Conklin (1912) with hypertonic sea water and existing in a common cytoplasm, one underwent a dexiotropic division and the other a leiotropic division whereas, normally, the orientation of the two spindles should have been equal.

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** Numbers given in the margin indicate pagination in the original foreign text.

Its analysis was attempted from two different viewpoints. Primarily, /162
studies like those by Penners (1923-1924), or by Tyler (1930) demonstrate its intimate relationship with morphogenesis; these authors actually demonstrated that the equalization of the normally unequal first division checks the development by inducing the formation of double monsters (duplicitas).

These results can be interpreted in two different ways. Either the equalization of division is the primary factor having affected the constitution of the ovum or, conversely, a modification of the morphogenetic agents has influenced the mode of division in a secondary manner. From this, one could assume that there is a cause and effect relationship between constitution of the ovum, its germinal localizations, and unequal divisions. Specifically Schleip, on the basis of his investigations with Clepsine (1914), made an attempt in his "determination of primitive evolution" (1929) to correlate the unequal segmentation with the presence of "polar plasms" which have an undoubted morphogenetic value. Nevertheless, Leopoldseder (1931) destroyed the vegetative polar plasm of the Clepsine ovum and thus produced an abortion of the germinal bands and provoked death of the embryo at the stage at which its essential elements should have differentiated; nevertheless, the segmentation remained normal. Despite the fact that this proves the morphogenetic value of the polar cytoplasm, its presence is by no means necessary for ensuring a normal course of mitosis. An analysis of the links existing between constitution of the ovum and its mode of division thus is far from being accomplished.

What is another attractive point for studying unequal segmentation is the fact that this segmentation can be modified by experimental intervention. For a long time, it has been observed - although sporadically and without systematic investigations - that various factors (cold: Crampton, 1896, Ilyanassa; compression: Browne, 1910, Cumingia; excessively high temperature or poor oxygenation: Penners, 1924, Tubifex) were able to induce equalization of the first mitosis which normally is unequal. In 1930, a paper was published by Tyler showing that compression, reduction in temperature, centrifugation and (to a lesser degree) heat and anaerobiosis, as well as irradiation by ultraviolet rays all produce an equalization of the first mitoses of ova of Chaetopterus, Nereis, and Cumingia. The great merit of this work is to extend to Polycheteae and /163
to Mollusca the results obtained by Penners (1923-24) in the Oligocheteae Tubifex, which we mentioned already above: Equalization of the first mitosis runs parallel with the formation of duplicitas. Obviously, the mechanism of inequality itself - or of possible equality - of the division has preoccupied the American author much less. We make all possible reservations as to his interpretation of the equalization of mitosis. The experimental facilities which he used would, according to his statement, effect a displacement of the spindle from its normal orientation, in which its major axis is located in a plane where the constitution of the cytoplasm is asymmetric. For example, a rotation through 90° would shift the axis of the mitotic figure into a plane in which the structure of the ovum is symmetric and, because of this very fact, the division would be equal. This explanation might at most be valid for cases of compression of the ovum between slide and cover glass at the instant of mitosis but certainly not for any other case. In fact, without knowing Tyler's results which, in addition, had not been published at the time of our experiments, we were able to observe during a previous study (1931) that an irradiation of the ovum of Lamellibranchia Barnea (Pholas) candida with a mercury vapor lamp provokes

equalization of the divisions; we were able to make a systematic study on this point from which we concluded that the obtention of equal segmentations is possible not only by intervening at the moment of mitosis but also during the entire course of maturation and even on the virgin egg. We are in a position to add at present (awaiting a subsequent publication) that this is true also for centrifugation, chilling, increase in temperature, and influence of certain narcotics. Manifestly, it is impossible to reduce all these factors - and specifically if they are effective at precocious stages - to a simple mechanical deviation of the position of the spindle. The modifications involved are certainly more profound, and their determination would bring considerable clarification as to the actual causes of unequal segmentations in Spiralia. This was the main incentive for continuing our research.

For reasons of convenience, we primarily investigated the first division. The results obtained from irradiation of the Barnea ovum showed that the in- /164
equality of the other mitoses presumably are due to a similar mechanism: Provided that one intervenes at the desired moment, the ultraviolet radiations will equalize the divisions of the second and of the third cycle as well as those of the first.

During a stay at the Zoological Station of Naples, we had an occasion of experimenting on ova whose unequal segmentation presented features that differed greatly from the usual type (of which Barnea is an excellent example). These specific cases are of dual interest: Primarily, one should determine the manner in which these cases fit the general framework, meaning that one could hope to define the fundamental causes of unequal segmentation; secondly, their very peculiarities may demonstrate certain factors or certain aspects that usually are masked.

If, from some viewpoint, this type of research may appear incomplete and leave some rather puzzling facts unexplained, its essential objective nevertheless has been reached. We were able to perform these investigations thanks to the generous support by the University Foundation of Belgium whom we wish to thank again at this point. We also express our gratitude to the Director Dohrn and to the Dean Ranzi for their amiable reception and for facilities made available to us.

1. Effect of Ultraviolet Rays and Centrifugation on the First Division of the Aplysia Limacina Ovum

A character highly specific for the inequalities of division in Spiralia is their remarkable constancy in the normal development of a species predetermined but frequently varying from one genus to the other in the same family, as has been observed for Polycheteae and Gasteropoda: The first division is sometimes unequal and sometimes equal or, at times, even skirts the limits of inequality and equality, as is the case of Serpulidae or of certain Nudibranchia. The /165
size of the ovum and its content of vitellus can just as little be invoked as basis for a possible systematization as the zoological affinities. Conversely, the first mitosis is always equal in Nemertinea and always unequal in the other groups of Spiralia: Hirudinea, Oligocheteae, Sipunculoideae, Gephyrea, Rotifera, and Lamellibranchia. In the latter groups, as well as in the Polycheteae and

prosobranchial gastropods, the coarse blastomere of the stage II is always the cell CD from which emerges the quadrant D representing the strain of the mesoblastic progeny. Nevertheless, in certain Opisthobranchia, the size ratio of the two first blastomeres is reversed (see Pelseneer, 1911), with the AB (purely ectoblastic and endoblastic) surpassing the cell CD. In this respect, we could mention Aplysia whose cell lineage has been thoroughly studied by Carazzi (1906). Might it be possible that the predominance of size of the cell AB in the case of Aplysia is determined by the same mechanism as in the conventional case with purely quantitative differences or may its determinism be entirely distinct?

This particular aspect of the question of unequal segmentation might be solved by subjecting the ovum of Aplysia to agents that reduce the inequality of the ova with blastomeres of dominant CD. This is exactly what we had in mind.

The Aplysia ovum, after internal fertilization, is spawned into small capsules interconnected by a long filament which the animal attaches to the algae and walls of the aquarium. Since the laying process is very slow, it is not difficult - with some supervision - to harvest the eggs immediately on emergence from the ducts. The envelopes, which harden on contact with the water, are still sufficiently soft at this particular moment to permit, by simple laceration, the liberation of a large number of ova without damaging them. After removing the small number of debris resulting from this operation, a culture of completely naked ova is obtained whose development remains just as normal in contact with the sea water as in the maternal matrix. The large species Aplysia limacina is more suitable than others for this operation, for which reason we used only this species in our experiments.

To properly understand our discussion, it is useful to give a few details on the normal course of development of the Aplysia ovum up to the stage II, /166 although there is not much one can add to the statements made by Carazzi in his paper of 1906.

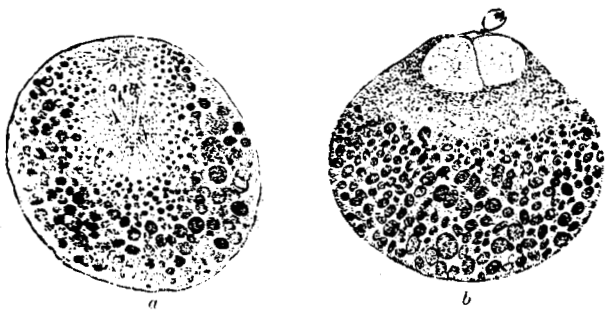

Fig.1
a - Microsection of an ovum at the instant of expulsion of the first polar globule; b - Section of an ovum at the instant of coalition of the pronuclei.

The ovum is rather voluminous and rich in vitellus. Its development is extremely slow. At 19°C (the ova were kept at this temperature by circulating the water in which the recipients were immersed), the first polar globule was expelled only 2 hrs after the laying and the second globule a good half hour thereafter; onset of the first segmentation mitosis was observed only 2 hrs after the end of maturation*. At the beginning of this relatively long delay, the ovum undergoes a highly characteristic modification. During the entire maturation process, the ovum remains

* For footnote see following page.

completely opaque and the animal pole is just about discernible by a slightly lighter spot. The microsection shown in Fig.1a (fixation: Allen; staining: ferric hematoxylin, eosin, light green) shows that the maturation figure is always surrounded by vitellus. However, after expulsion of the second polar globule, at a stage corresponding to opening of the sperm-aster, almost the entire animal half of the ovum becomes separated (Fig.1b). The aspect of the ovum in vivo is extremely characteristic, with the clear pole standing out clearly against the greenish vitelline conglomerate of the vegetative pole. Between the two, there is a transition zone occupied by small gray granules.

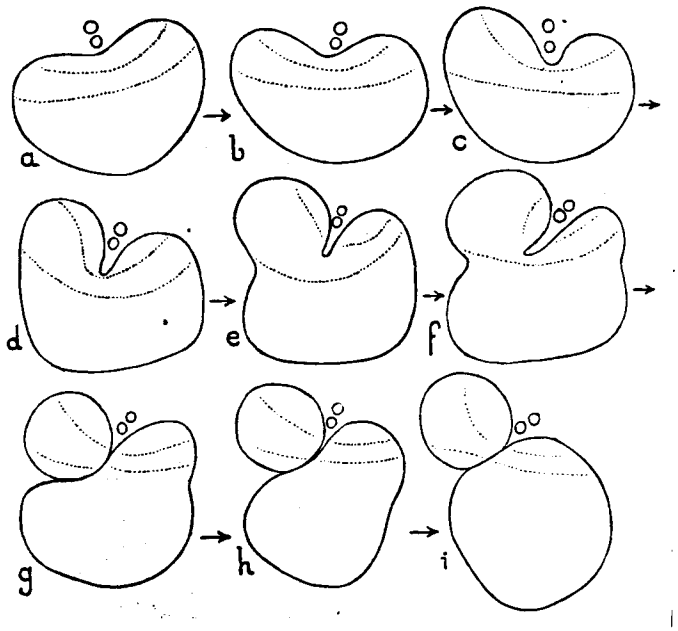

Fig.2 Successive Stages of a First Normal Segmentation, Traced in Vivo.

This stratification of substances, taking place after the end of maturation, is encountered in numerous forms of Spiralia. Spek (1930) drew attention to this phenomenon with respect to the ovum of Nereis and attributed considerable importance to it. It is exactly at this same stage that, in Oligocheteae and Hirudinea, the two polar plasms - animal and vegetative - assemble again, constituting two plasmic conglomerates that seem to play a highly important role in the development. /167

The cytodieresis starts with an elongation of the ovum in the equatorial direction; this is followed by an anlage of the groove starting from the animal pole (Fig.2a); until now, it is impossible to predict which blastomere will have a larger size. However, as shown in the sketches b and c of Fig.2, the ovum soon tapers on one of the sides of the shallow groove and forms a sort of prolongation which becomes rounded and individualized; in fact, the groove which originally seemed to divide the ovum into two equal halves becomes highly oblique and deviates more and more, finally isolating the small blastomere CD, a process that is even more pronounced since, facing this, a fissure soon appears at midheight between the vegetative and the animal pole (Fig.2d, e, f). From then on, the two cells only have to isolate themselves and become rounded (Fig.2g, h, i). In fact, the two last sketches show a peculiar property: The animal portion of what will become the blastomere AB has undergone more or less /168

* Let us mention, in passing, that polycentric divisions of the second maturation are not uncommon in some spawns and even interfere because of their frequency of occurrence. Such divisions are due to a retention of the first polar globule and furnish an explanation for the polycentric mitoses of the first segmentation, mentioned but inadequately interpreted by Carazzi.

the same transformations although with a considerable lag; one can even observe (Fig.2f, g, h) a very slight fissure symmetric to that which isolates the CD and which thus seems to pass through the very center of AB. Nevertheless, this deformation is purely transitory. The staggering of chronology existing between the two halves of the animal pole, of which one forms CD and the other passes over into AB, is not always as distinct. In certain less frequent cases - as indicated in Fig.3 - there occurs formation of an actual trefoil stage in which two spheres of animal substance are isolated at the summit of a coarse vitelline lobe. The resemblance with the corresponding stage of Ilyanassa, Dentalium, or Myzostoma is rather striking; nevertheless, this is only superficial. In fact, as demonstrated in the historical experiments by Crampton (1896) on Ilyanassa and by Wilson (1904) on Dentaliidae, the ablation of the polar lobe (which passes, incidentally, into CD) leads to a total mesoblastic deficit. Recent experiments by Hatt (1932), on the annelid Sabellaria yielded similar results. However, the coarse vitelline lobe in Fig.3 is by no means a homolog of the polar lobe; it passes into the cell AB all of whose derivatives, in accordance with the detailed cell lineage established by Carazzi (1906), are endoblastic or ectoblastic.

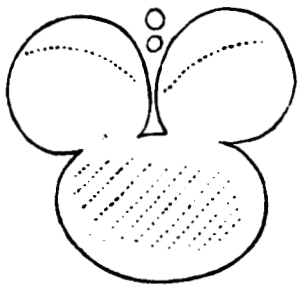

Fig.3 Other Type of Normal Segmentation, Showing a Polar Pseudolobe.

* * *

We subjected cultures of segmented Aplysia ova to radiation from a mercury vapor lamp. The results obtained were exactly the same as with Barnea; the first division became equal. Nevertheless, there exists a difference of /169 degree between the results obtained in the two species. Whereas, in Barnea, the transformation - in the vast majority of cases - was completed at first onset, numerous forms of transition were observed in Aplysia: The two blastomeres do not have the same size, with AB being smaller and CD being larger than normally. This form of mitosis which, for convenience, we will call "subequal" division is especially frequent in batches affected little by the irradiation, either because of the fact that the dose was low or the stage rather insensitive (as we will explain in more detail later in the text). The manner in which an ovum of unequal segmentation divides is particularly instructive. Whereas, in the case of equal division as that shown in Fig.4, the groove that had remained symmetric progressively penetrates through the ovum, starting from the animal pole and finally joining a notch formed exactly at the vegetative pole, the processes in the case of a subequal division are more com-

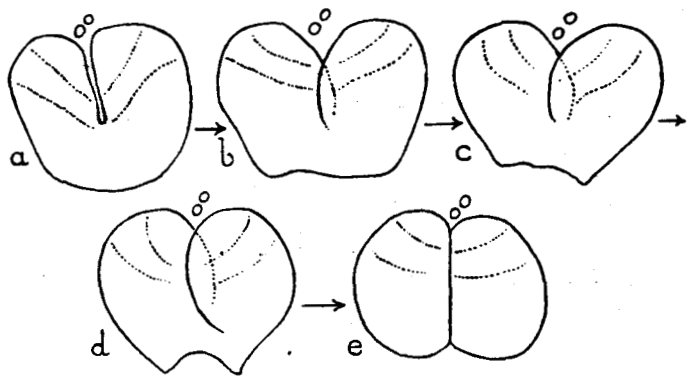

Fig.4 Successive Stages of an Equal Segmentation, Traced in Vivo.

plex. Figure 5 gives a first example, showing that the beginning of cytodieresis starts normally: There is formation of the characteristic notch midway between the two poles, with nothing permitting at this time to assume an abnormal issue (Fig.5a). However, in the vicinity of the vegetative pole in the midst of the vitellus (Fig.5b), a second notch is formed which, very soon, takes the path of the first one and leads to a definite separation of the two blastomeres (Fig.5c). This is followed by rounding of the blastomere CD, leaving no trace of this sort of tentative normal division. /170

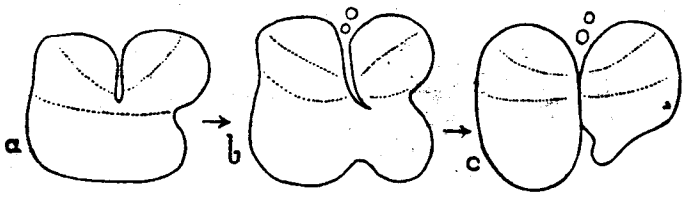

Fig.5 Sketch Made in Vivo, Showing the Successive Aspects of a Subequal Division.

Figure 6 gives a slightly different modality. Here again, the start of division begins normally (Fig.6a, b, c). However, very soon thereafter, the vegetative pole shows some activity, thinning to the point of clearly showing the intercalated vitellus granules; the pole is the seat of numerous foldings (similar processes are frequently encountered during an equal division but never in a normal segmentation). At the same time (Fig.6d, e), the diaphragm which seems to constrict what should originally have formed the blastomere CD progressively regresses toward the vegetative pole; in the vicinity of the latter, the two blastomeres finally separate. In the above-described case, the disappearance of the equatorial notch has been more premature than in the example shown in Fig.5.

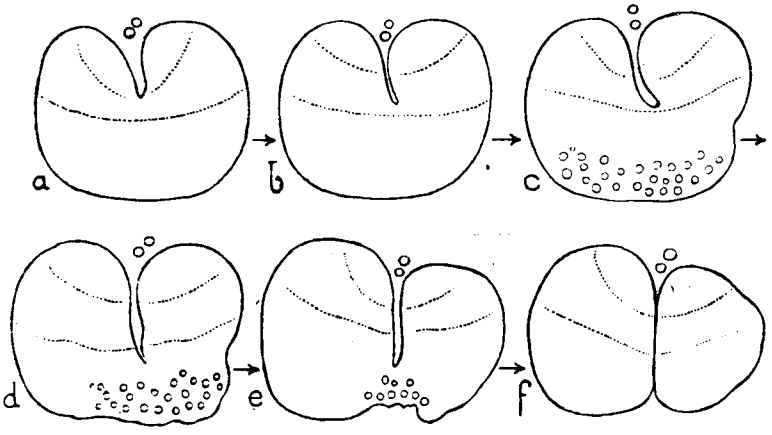

Fig.6 Subequal Division; Different Modality; Sketched in Vivo.

These detailed descriptions make it possible to state that, at the moment at which an unequal division takes place, the factors inducing normal inequality are by no means absent and not even retarded (since the beginnings of division take place in the conventional manner), but they do seem somewhat weakened. /171

A cytological examination showed no modification of the spindle which, in addition, always remains localized close to the animal pole, with the aster fibers never penetrating into the vitelline zone. The equalization of division, as we had postulated for the Barnea ovum, thus reveals essentially cytoplasmic modifications.

On subjecting the ova at various stages, ranging from fertilization to segmentation, to equal doses of irradiation, we were able to demonstrate a progressive increase in the number of equal segmentations of Barnea. This equalization continued in the subsequent divisions and was accordingly greater the more the time of irradiation was retarded. Nevertheless, at a given moment, the normal course of unequal segmentations regained the upper hand. From this, we concluded that the modification caused by the irradiation was only transitory. The return to the normal course of division, which we always observed, obviously has more chance of happening before the first mitosis the more the intervention had been premature. We thought that this factor most likely is sufficient for explaining the ascending trend of the curve of equal segmentations before the first mitosis, the more so since the highly regular slope of this curve (see Fig.3 of our paper of 1931) obviously precludes any interference of multiple factors.

The results obtained with Aplysia are different. We repeated numerous times and with various spawns, the experiment of irradiating - in exactly the same manner - batches taken from successive stages. In all cases we observed a distinct maximum of action near the instant of expulsion of the second polar globule. As a typical example, we are giving the detailed results of one of these experiments:

Experiment A. The hours are indicated, starting from the first spawning. Irradiation was given each time under exactly the same conditions and of the same duration (1 min).

Batches	Hours after Spawning	Stage
1	40 min	
2	1^h35^m	Expulsion of the first polar globule between 1^h20^m and 1^h30^m.
3	2^h17^m	
4	2^h55^m	Expulsion of the second polar globule between 2^h40^m and 2^h50^m.
5	3^h45^m	
6	4^h40^m	
7	5^h25^m	First division starts at 5^h30^m and ends at 6^h30^m.

/172

In Fig.7, the ordinate gives the percentages of equal segmentations obtained at these different stages; the abscissa shows the time from the start of spawning as well as the moments of irradiation and the main stages. The solid curve represents the total number of equal divisions, obtained by counting /173 a strictly equal division as 1 and a subequal division as 1/2; the dashed curve represents only equal segmentations and the dotted curve only subequal divisions. It is obvious that the latter are specifically abundant at moderately sensitive

stages; evidently they disappear as soon as the sensitivity diminishes but they also do so when it increases making way for strictly equal divisions. The curves for equal segmentations show a distinct bell shape, with the maximum extending over the entire period encompassing the expulsion of the second polar globule.

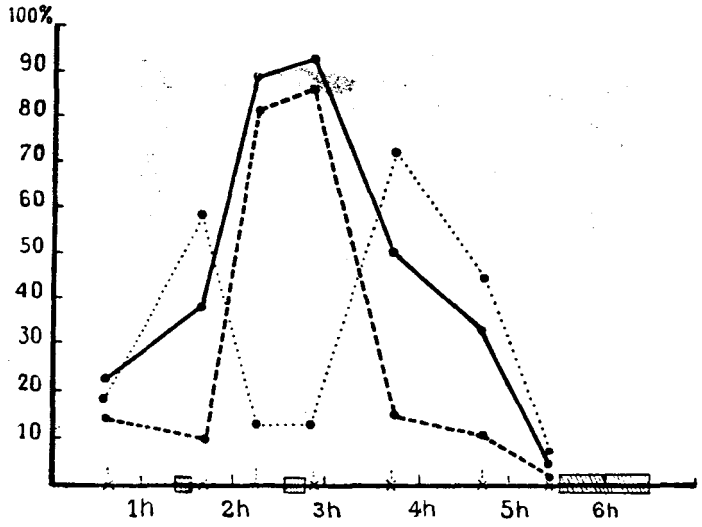

Fig.7 Curves Showing the Percentage of Total Equal Segmentations (Solid Line), Truly Equal (Dashes), Subequal (Dots) for the Same Ultraviolet Doses but at Different Stages. The abscissa shows the hours after spawning; the stages are given as follows: first polar globule, second polar globule, segmentation indicated by segments; moments of irradiation, indicated by crosses.

All other experiments are in strict accordance with this one. Figure 8 represents two curves (equal 1; subequal 1/2) obtained in the same manner from another spawn but with two irradiation series of different duration (one of 30 sec and the other of 2 min: experiment B). Their agreement with the solid curve is very satisfactory.

Thus, a maximum of proved effect exists at the end of maturation. How can this be interpreted? Must one assume an overall increase in sensitivity of the ovum to ultraviolet radiation; if this total sensitivity does not vary, might the factors regulating the inequality of division be specifically susceptible to be modified at this particular stage?

The first of these hypotheses is the one to be retained, for the following reason: As demonstrated before, the development of the Aplysia ovum is very slow and is considerably retarded under the influence of irradiation. It is true that the expulsion of polar globules is only slightly retarded but the period separating the maturation from the segmentation is particularly protracted. This paralysis is obviously more pronounced the stronger the ultraviolet irradiation dose had been. However, in the experiments mentioned here, we found a highly satisfactory agreement between the retardation of segmentation and the number of equal divisions. This can be demonstrated in the following manner: The slowness of division makes it possible (restricting the procedure to an approximate percentage referring to about 50 or 100 ova) to rapidly count the segmented ova in any batch at a given moment. The resultant curve does not have strict validity but is sufficiently approximate for reflecting a general course; the slope is more shallow the more the segmentation had been retarded. Figure 9 shows two of these curves (solid lines) obtained, respectively, 5^h55^m after spawning (10% of divisions in the control) and 35 min later (control, 80% of divided ova) during the experiment A. Compared with the curve (dashes) of /174

normal segmentations (i.e., the inverse of the total curve of equal segmentations in Fig.7), it is easy to see that their general slope is the same. /175

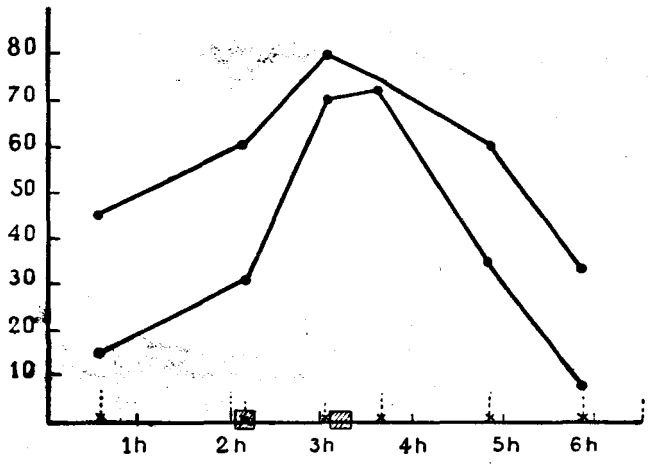

Fig.8 Two Curves of Total Equal Segmentations for Two Different Irradiation Intensities.
Legend same as in Fig.7.

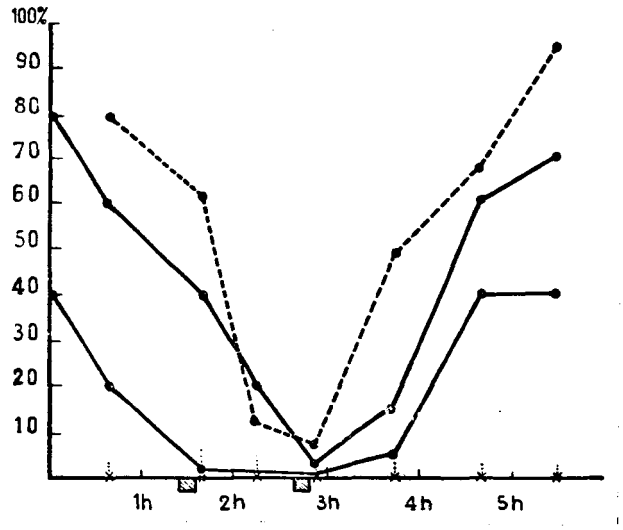

Fig.9
Broken line: curve of normal segmentation; Solid line: two curves expressing the number of segmented ova counted at two different times. Same legend as for Figs.7 and 8.

The results of other experiments are entirely comparable.

This increase in sensitivity of the ovum to a physical agent, at a given stage, is of some interest. The sensitivity again reflects these changes in the constitution of the ovular cytoplasm which had been demonstrated specifically in investigations such as those by Herlant (1920) or by Runnström (1928). Apparently, achievement of maturation represents a true critical stage, at which instant the cytoplasmic modifications must be greatest. In Barnea candida, it is exactly at this moment that the cortical reactions to certain fluctuations in the salt balance of sea water change abruptly (Pasteels, 1930).

* * *

As still another experimental means susceptible to affect the form of the first division, we made use of centrifugation. In fact, ever since the studies by Tyler (1930), we know that this operation leads to an equalization of the mitoses of various Spiralia ova (Chaetopterus, Nereis, Cumingia). Naturally, in these two annelids and the one lamellibranch, the size of the blastomere CD predominates. Despite its specific type of division, the Aplysia ovum reacts in the same manner: The centrifuged batches showed a relatively large number of equal or subequal stages. Nevertheless, these transformations were less pronounced than after irradiation. We were never able to obtain a batch completely devoid of normal segmentations; in addition, the number of subequal segmentations always exceeded that of equal segmentations. On the other hand, the stage

at which the ovum is centrifuged seems of little influence; at least, we never were able to record variations in sensitivity of a systematic form, as we had encountered above in ultraviolet irradiations.

On removal from the centrifuge, the ova show the characteristic aspect which had been described at numerous occasions: the vitellus and the fatty /176
matter, accumulated at the two opposite poles, leave a transparent narrow strip of cytoplasm free. The position of the polar globules is highly variable with respect to the centrifugation axis; on the other hand, in the case of centrifuging during the second maturation mitosis, the site of expulsion of the second globule coincides with that of the first; from all observations, the real polarity of the ovum remains intact and the ova become oriented in an arbitrary manner within the centrifuge.

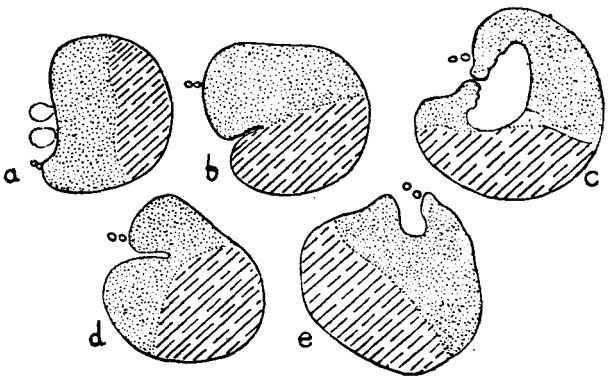

Fig.10 Sketch Made in Vivo of Lobulations Occurring in Centrifuged Ova. Dotted area: fatty calotte; hatched area: vitellus.

The abnormal distribution of substances might have had a direct influence on the direction of the achromatic spindle or on the direction of the cytodieretic groove, as had been observed - for example - in experiments made by v.Parseval on Tubifex (1922). An unexpected circumstance placed this point out of the question. Actually, although ova centrifuged for some time gradually permit invasion of the intermediate zone by vitelline and fatty granules, this process is very slow and never leads to a normal image: Fatty matter and vitellus come into contact but do not mingle. We have seen above that in normal development a short time after end of maturation, the vitelline substances accumulate toward the vegetative pole leading to a characteristic accentuation of polarity: it is at this particular moment that an abrupt and sudden modification of the centrifuged ovum takes place, making it impossible to distinguish it from a normal ovum. No matter at which stage the ovum has been centrifuged, irrespective of the duration of centrifugation, irrespective of the aspect assumed by the stratification, and no matter whether the transparent median zone is still distinct or has already disappeared, this return to normal aspect by the ovum takes place always at exactly the same stage. The transformations of the ovum taking place at this stage thus are radical and the processes that accentuate the polarity in normal developments initiate forces capable of re-establishing the normal distribution of inclusions which some extraneous influence had profoundly modified.

Although ova centrifuged during or a short time after maturation return to a normal aspect, it is quite difficult to modify the distribution of substances after this stage, which no doubt is due to the great increase in viscosity produced by exhaustion of the sperm-aster or formation of the spindle. Conse- /177
quently, equal division - or subequal division - takes place in ova that are

normal from the viewpoint of distribution of visible structures. Thus, this cannot be considered a causative factor, which is even more applicable to a possible modification of the location of invisible hypothetical substances, at least under the direct effect of centrifugation.

Centrifugation itself does not lead to retardation of development; quite the contrary, it is not rare in centrifuged batches to see divisions precede (although by very little) that of normal batches.

Finally, still another feature again reveals the special state of the cytoplasm shortly after expulsion of the second polar globule. In all centrifuged batches, irrespective of the moment of centrifugation, always the same stage is encountered, i.e., shortly after the end of maturation a certain number of ova appears whose cortex shows a considerable activity. Most often, at the animal pole and sometimes also at the point of separation between fatty matter and vitellus, small superficial lobules are formed or else a deep groove appears propagating through the ovum and, in certain cases, succeeding in dividing this ovum into two pseudoblastomeres of which only one, namely the nucleated type, will further divide. Figure 10 shows a few aspects, sketched from the living organism.

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/178

It seemed of interest to continue this analysis by combining irradiation and centrifugation. General circumstances have permitted only a certain number of experiments, which is the more regrettable since the results were not only peculiar but even paradoxical.

Experiment C. Two series of batches of the same spawn, one intact and the other previously irradiated, were centrifuged at the same time and in the same manner. Since the development was particularly slow, the ova segmented only during the night and the segmentation could not be observed in close detail. The next morning, the various batches were at the following stages:

Controls	Irradiated Ova	Centrifuged Ova	Irradiated and Centrifuged Ova
-	-	-	-
XVI blast.	II and IV blast.	XVI blast.	VIII and XVI blast.

Thus, the evolution of ova which had successively been subjected to the effect of irradiation and centrifugation approached much more closely the normal development than that of ova subjected only to irradiation.

The possibility of an experimental error can be excluded; we gave a single-dose irradiation to a relatively abundant culture from which we previously had taken the batches to be centrifuged later. This harvesting was done in two processes at two different stages, with the results agreeing perfectly.

Experiment D. The ova were harvested at the moment of spawning: culture a. Twenty minutes later, during the first maturation mitosis, one portion was irradiated for 3 min: culture b. At three different times, during the first maturation mitosis and then shortly before expulsion of the first polar globule and

during the second maturation mitosis, the batches of a and b were collected and centrifuged at the same time for $2\frac{1}{2}$ min at 3250 rpm. Let us denote these batches, respectively, by a1, a2, a3, for the normal types and by b1, b2, b3 for the irradiated types. At a time 6^h15^m later, the batches of the series a had segmented; below, we give the number of divisions observed in the series b: /179

b	4 %
b1	27 %
b2	53 %
b3	3 %

and, 40 min later,

b	14 %
b1	77 %
b2	90 %
b3	15 %

The equal divisions were counted in accordance with the completion of mitoses in the various batches. The results were as follows:

	Normal Divisions	Subequal	Equal	Total Equal 1 + Subequal ½
a	93.7 %	3 %	3.3 %	4.8 %
a1	67	11	22	32.5
a2	54	17	29	37.5
a3	78.5	11	10.5	16
b	40	25	35	47.5
b1	81	9	10	14.5
b2	81	7	12	15.5
b3	54	19	27	36.5

The batches a3 and b3 showed a slightly peculiar behavior: The first batch exhibited an abnormally low number of equal segmentations (after centrifugation of normal ova); the second batch b3 (centrifugation of irradiated ova) showed a number of equal segmentations closely resembling the number encountered in only irradiated ova (b). However, these two batches were centrifuged simultaneously. Because of fluctuations in the electric power, it was quite difficult to keep the electric centrifuge always at the same speed, so that most likely an error of technique might explain the aberrant behavior of these two batches; in any case, the fact that, after exactly the same centrifugation, agreement exists between a weak effect on the division of normal ova and the correction of irradiated ova constitutes a satisfactory indirect proof. Centrifugation performed after irradiation compensates the effects of this irradiation, both from the viewpoint of retardation of development and equalization of cytodieresis. Consequently, this corrective effect seems to cover the general action of ultraviolet rays rather than their specific effect on the mode of division. From this, it can no doubt be concluded that ultraviolet irradiation and centrifugation, taken separately, will equalize the division over different pathways. /180

The mechanism of these centrifugation effects* remains even more puzzling

* For footnote see following page.

since we were forced to interrupt this research and to be content with fragmentary data. For example, we could have continued the study by determining the critical dose at which centrifugation manifests its effect on previously irradiated ova and by defining whether this threshold does or does not agree with the threshold of action excited on the segmentation of ova that had not been subjected to ultraviolet radiation. On the other hand, it would have been of some interest to make the opposite experiment, namely, that of irradiating the ova after previous centrifugation.

* * *

These experiments on the Aplysia ovum furnish no new data as to the actual mechanism of unequal segmentation. This point can be picked up only later; however, we would like to counteract here a possible objection. Certain treatments that have an equalizing effect on the segmentation (for example, ultraviolet radiation) will retard the course of development. Could this equal segmentation possibly be merely an ordinary effect of dyschronism? In fact, determination of the inequality of division could actually be more retarded than the mitotic processes themselves. In such a case, this experimental production of equal divisions obviously would be of only mediocre analytical value.

Let us refute this objection by stating first of all that we have to do here with a species in which equalization of division has been obtained by irradiation and centrifugation whereas during the maturation there were definite signs proving that, from this stage on, the factors of unequal division were already present and active. This concerns specifically the Nereis ovum. These experiments were made by Tyler (1930); on the other hand, from work done by /181
Bonnevie (1910) and F.R.Lillie (1912) we know that, during the second maturation mitosis, the sperm-aster is subdivided and that the two resultant asters remain united by a "central spindle" but are of unequal size. Bonnevie's concept that the inequality of cell division was due to an inequality of the mitotic centers has been refuted at numerous occasions (see Schleip, 1929). This leaves only one possible and probable interpretation: The inequality of the two centers of the sperm-aster reveals the asymmetric constitution of the cytoplasm. The causes of inequality of division are present already at the end of maturation, and experimental interventions only succeed in modifying them but not in retarding their appearance.

In our own experiments, we have encountered arguments that might refute a possible action of dyschronism. With respect to ultraviolet radiation, we insisted on the fact that the beginning of a subequal division closely resembled a normal division and that, consequently, the factors of unequal division were neither absent nor retarded but merely weakened. This is exactly the same for centrifugation in which, in addition, dyschronism is much less probable since the normal rhythm of the development is not affected.

We can conclude from this that the specific case of Opisthobranchia ova

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* We should mention that centrifugation is far from being an anodyne treatment since it changes nothing in the properties of the ovum. Nevertheless, centrifugation is used in numerous studies for measuring not only the comparative but also the absolute viscosity (Heilbrunn, 1928). Can one be absolutely certain that centrifugation does not affect the viscosity?

with a small CD blastomere can be fitted into the usual framework of unequal segmentations of Spiralia ova; the answer here is definitely positive. The mechanism of inequality is qualitatively the same and, no doubt, only quantitative differences cause a displacement of the equilibrium in one or the other sense, at times leading to predominance of the CD blastomere and at times to that of AB.

Resumé

1) The ovum of Aplysia limacina, just as in the case of numerous Opisthobranchia presents a stage II with a small blastomere CD and a large blastomere AB. We intended to determine whether this inequality of particular character could be reduced in the same manner and by the intervention of the same agents as the conventional type of the unequal stage II (CD > AB) of Spiralia ova. /182

Here again the answer is positive: Under the influence of irradiation by mercury vapor lamps and of centrifugation, the first division becomes equal.

2) The action of factors such as centrifugation and ultraviolet irradiation on the cytodieresis is still unexplained. Nevertheless, it most certainly cannot be attributed to simple dyschronism. Rather, such agents modify a predetermined cytoplasmic state, so that they can be attributed to an analytical value.

3) During our experiments, we observed a certain number of facts demonstrating that profound modifications are taking place in the ovum shortly after the end of maturation. These observations are as follows: a) At the instant of expulsion of the second polar globule, the sensitivity to ultraviolet radiation is maximal; b) a short time after maturation, all centrifuged batches (irrespective of the moment or intensity of centrifugation) show - always at the same stage - more or less accentuated lobulations; c) somewhat later, at the moment of opening of the sperm-aster, a reflux of vitellus toward the vegetative pole and a liberation of the animal pole are observed in the normal development. It is always at this stage that all centrifuged ova - no matter of what type - return to a completely normal aspect, leaving no trace of sedimentation of the substances that had appeared on removal from the centrifuge.

2. Significance of Polar Plasma; Centrifugation and Irradiation of the Myzostoma Ovum

The division of the ovum with formation of a polar lobe is a modality of unequal segmentation which, because of its strangeness, has for long intrigued the observer. This modality takes place sporadically in various groups of Spiralia: Polycheteae (Chaetopterus, Sabellaria, Myzostoma), scaphopodic Mollusca (Dentalium), Gasteropoda (Nassa, Ilyanassa), and even Lamellibranchia /183 (Ostrea virginica, according to older observations by Brooks, 1880). The differences between an unequal segmentation of the usual type and those of an ovum with polar lobe are striking at first glance: In one case, it is the eccentric position of the achromatic spindle which seems to rule the unequal distribution of the cytoplasm while in another case the spindle remains central but a coarse

vegetative lobe separates at the same time as the two blastomeres and rejoins one of these near the end of the cytodieresis. Nevertheless, the result or, one could say, the function of these two different modes is identical: primarily, a preponderance of CD, then of the quadrant D, strain of somatoblasts. In addition, a type of transition exists, such as takes place - for example - in Chaetopterus*: The polar lobe is highly reduced but the achromatic spindle is distinctly eccentric.

The peculiar characteristic of various ova with polar lobe is the existence of "plasms" at the vegetative pole. These are accumulations of dense cytoplasm, totally devoid of vitelline inclusions. Except in Oligocheteae and Hirudinea, which were specifically studied by the school of Schleip, no characteristic "polar plasms" are encountered in ova with direct unequal segmentation. In fact, it seems quite difficult, in accordance with Schleip (1929), to detect the equivalent of animal and vegetative polar plasms of Hirudinea and Oligocheteae in all Spiralia. With respect to the vegetative plasm, Schleip - based on work by Morgan (1910) and Browne (1910) on Cumingia as well as by Vejdovsky (1881) on Sternaspis - assumed its existence in these two species which both exhibited direct equal segmentation. Nevertheless, when reading the papers by Morgan and Browne, it is quite evident that the "plasm" of Cumingia is nothing but a superficial difference in pigmentation. On the other hand, we had an occasion to examine ova of Sternaspis in vivo and in microsection. The unfertilized ovum presents actually a cytoplasmic collection devoid of vitellus at the vegetative pole, at the site that just has separated from the hilus; however, from the /184
beginning of maturation, this plasm definitely disappears without leaving a trace.

The existence of an animal cytoplasm in Spiralia other than Hirudinea and Oligocheteae is even more contestable. It is true that, in numerous annelids and mollusks, the animal pole separates from any inclusion at the end of maturation. Nevertheless, although this process most likely reveals an important physiological transformation, there is no justification to compare this with the formation of animal plasm in Hirudinea-Oligocheteae. In fact, although this latter obviously passes into the blastomere CD, then into the blastomere D, and finally into the two somatoblasts 2d and 4d, nothing similar takes place with respect to the animal cytoplasm of the other Spiralia. It is sufficient to study the formation of the stage VIII in a Nereis ovum (for example) to see that this cytoplasm of the animal pole passes over into the first tetrad of micromeres.

In summation, one might thus state that Spiralia ova present a highly special case (Hirudinea, Oligocheteae) where two well-differentiated polar plasms, one at the animal pole and the other at the vegetative pole, both pass into CD, then in D, and finally into the strain cells of germinal bands. In the other groups, if a polar plasm exists at all, it is always of the vegetative type and always is accompanied by formation of a polar lobe at the instant of

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* This case is by no means isolated. At the Naval Station of Concarneau we made experiments in 1932 with ova of the Polycheteae Nerine. Fertilization was quite difficult here; nevertheless, the few ova that segmented at all did so in the same manner as the Chaetopterus.

first divisions.

The characteristic cytoplasm, however, is not encountered in all ova with polar lobe. The plasm is extremely distinct in Dentalium (Wilson, 1904) and in Myzostoma (Wheeler, 1898), is still well visible in Chaetopterus (F.R.Lillie), and is absent in the large vitelline ovum of Ilyanassa (see diagrams in the recent paper by Morgan, 1933); Fauré-Fremiet (1924) mentioned none in his Sabellaria; the Ostrea ovum would have to be re-studied in this respect.

In contrast to the cytoplasms of Hirudinea and Oligocheteae ova, which differentiate after achieving maturation, those of Dentalium and Chaetopterus preexist in the virgin ovum. We also had occasion to make detailed studies of the Myzostoma ovum (glabrum and cirriferum); since our observations do not agree entirely with the previous descriptions, we will discuss them in some detail.

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/185

At the vegetative pole, the virgin ovum has an inclusion of greenish color which had originally been reported by Beard who interpreted it as a vitelline nucleus. Driesch (1896) successively encountered this substance in the polar lobe, in the blastomere CD, in the blastomere D, and finally - in part - in the first somatoblast 2d; he believed that the other part passed into the second somatoblast 4d. He attributed to this the property of a "preformed organogenic substance". The non-vitelline nature of this inclusion was recognized by Carazzi (1904). In his cytological study of the Myzostoma ovum, Wheeler (1898) detected at the vegetative pole of the oocyte I (with the ovum being fertilized at this stage) a granular zone distinctly differentiated from the spongy vacuolar cytoplasm that occupies the major portion of the ovum. In addition, Wheeler placed at this point a darker crescent, representing the green inclusion.

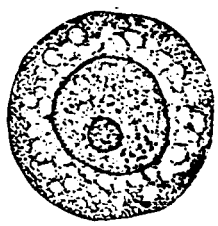

Fig.11 Microsection of an Oocyte I; Uniformly Trabecular Cytoplasm.

Despite the most careful examination (Allen fixation; triple staining with ferric hematoxylin, eosin, light green), we were unable to discover this structure for the oocyte I. As indicated in Fig.11, the ovum at this stage is of uniformly trabecular texture and the green substance can be discovered only in microsections. This aspect of the cytoplasm remains unchanged until the end of the first maturation mitosis. At this moment, as clearly observed by Driesch in vivo, the pink pigment, which until then had colored the egg in its totality, migrates to the animal pole. Let us specifically mention that this pigment becomes localized at the cortex and leaves the maturation macula intact, defining the formation of a large ring covering the upper half of the ovum. These transformations, seen in vivo, run parallel to a modification of the cytological aspect. The vacuolar cytoplasm shifts toward the animal pole and toward the cortex, leaving a granular cytoplasmic area at the center of the ovum which extends to the entire vegetative pole. In this respect, Fig.12 is entirely comparable to one of the sketches given by Wheeler. The subsequent evolution agrees well with the description /186 by this author: The two nucleated spheres of the trefoil stage are filled with vacuolar cytoplasm, with the entire granular plasm passing into the polar lobe.

Thus, it seems that the polar plasm, in Myzostoma, is not differentiated in the virgin ovum but gradually forms during the maturation process. This is the reason for the fact that the green substance pre-exists in the virgin ovum, provided that its vegetative vesicle does not constitute this cytoplasm. In fact, we were able to prove this point by subjecting the Myzostoma ovum to intense centrifugation. Apparently, the ovular structure is scarcely modified by this, while the green substance is shifted to some site in the ovum. The location of the red pigment and the ratios of spongy to granular cytoplasm remain intact; since the green substance cannot be detected on the microsections, the cytological figure of a centrifuged Myzostoma ovum will not differ from that of a normal ovum. Figure 13 shows that the segmentation does not change and that the green substance, for example, will pass close to the animal pole (Fig.13a) or will be distributed between the polar lobe and one of the two blastomeres (Fig.13b) or else will still be completely contained in AB (Fig.13c). Finally, we give a stage IV, in which this substance distributed between A and B (Fig.13d). Not only the segmentation but also the development of these ova remain completely normal. The organogenetic significance of this green inclusion thus is nil. Here we have to do only with a simple inclusion of either a fatty nature (which would readily explain its disappearance after application of conventional cytological methods) or of a pigmentary nature which would present simple coincidence relations with the polar cytoplasm of the vegetative pole. In this respect, this substance - which can be readily detected in the living organism - may serve as a highly useful topographic indicator.

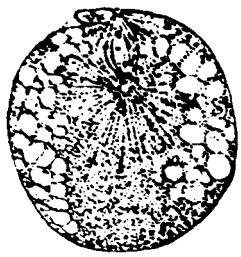

Fig.12 Microsection of an Ovum during the Second Maturation Mitosis. The trabecular cytoplasm has receded to the periphery, leaving the vegetative pole free.

/187

Thus, we can conclude that the polar cytoplasm, in Myzostoma, is built up during the maturation*.

* * *

We made an attempt to modify the mitosis of Myzostoma ova by subjecting them to agents susceptible of modifying unequal divisions of a direct type.

Above, we already mentioned centrifugation. Despite all our efforts, consisting in the most energetic intervention at widely differing stages, we were never able to obtain anything but normal divisions. This negative result is in contradiction to the equal divisions obtained by Tyler (1930) who applied this particular treatment to ova of direct unequal division of Nereis and Cumingia and to those obtained by ourselves (1933) in the ovum of the opisthobranch Aplysia (specific case: CD being smaller than AB). With respect to ova with polar lobe, the experiments by Tyler (1930) showed that the ovum of Chaetopterus

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* However, let us note that, in one spawn, we observed much more precocious differentiations, starting from the oocyte I. The red pigment conglomerates there in an extremely dense calotte at the animal pole instead of being distributed over the entire cytoplasm. However, the strong maturation (depolarization) and segmentation anomalies make one suspect that these ova are overmature.

is able to react in a positive manner and give equal segmentations, provided that it had been centrifuged at a well-defined stage (piriform ovum); we should /188 mention that the ovum of Chaetopterus represents a transition stage between the two types of mitosis. The ovum of Ilyanassa with a characteristic lobe seems just as refractory as our Myzostoma, since Morgan recently (1933) studied the effect of centrifugation of this ovum and was unable to find any instance of equal division.

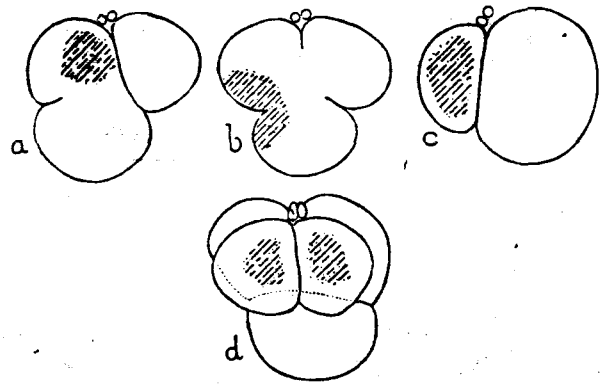

Fig.13 Centrifuged Ova, as Observed in Vivo.
a - Green substance at the animal pole; b - Green substance (hatched area) partially located in the polar lobe and partially in one of the two blastomeres; c - Ovum divided in two: green substance in AB; d - Ovum divided in four, with the polar lobe not yet fused to D, the green substance is distributed between A and B.

We also irradiated ova of Myzostoma with a mercury vapor lamp. Numerous ova, even at the strongest doses, remained entirely normal. Some ova, however, reacted by showing a tendency to equal segmentation.

At the moment of coalition of the pronuclei, in the normal development, the vegetative pole becomes flattened and subject to cortical tremors; the green substance seems heaped up there. This stage is equivalent to the piriform stage of Chaetopterus. Obviously, some peculiar event takes place in the ovum at this moment, presumably in intimate relationship with formation of the polar lobe. The ova, reacting to the irradiation, remain inert and rounded-off at this stage; they stop forming lobes and, at the moment at which the groove forms at the animal pole one can predict the formation of two equal blastomeres. As shown in Fig.14, the groove reaches the vicinity of the vegetative pole; it is even possible that a notch might form facing the main groove. This evolution, however, is extremely slow and the mitosis proper will never be completed. It seems that, at the very level of the polar plasm, an obstacle insurmountable to the groove exists which, after having remained for some time in situ or even having deviated laterally, will finally fall back on itself and leave the ovum unsegmented.

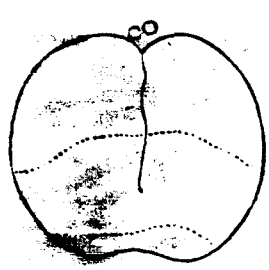

Fig.14 Irradiated Ovum, with Maximum Groove Extension and Undivided Ovum.

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Of two agents considered here, namely centrifugation and irradiation, the former is always the least efficient. For example, in the case of the Barnea ovum, it is extremely easy to obtain a culture presenting 100% of equal segmentations after irradiation by ultraviolet rays, whereas even the most intense /189 centrifugations will produce not more than 20% of positive results. In the case of Myzostoma, irradiation doses that cannot be exceeded without risking serious lesions of the ovum, modify the segmentation only in a

minority of ova; this makes it understandable that centrifugation remains completely ineffectual. Thus, the general sensitivity of the Myzostoma ovum with polar lobes to agents that produce equality of division is lower than that of ova of unequal division of the direct type. It is also true that the action of ultraviolet radiation, producing the equalization of divisions, inhibits the formation of the polar lobe. The two modalities of unequal division which, at first glance, seem to differ so widely actually react in exactly the same manner (but to differing degrees) with respect to one and the same experimental agent. This statement can only reinforce the hypothesis of an identical basic mechanism, diverging only by quantitative differences, as we had suggested above. It is rather unlikely that these differences have to do with chronology; as demonstrated above, the substance characterizing the polar lobe, i.e., the cytoplasm, frequently is differentiated starting from the virgin ovum; nevertheless, as in the case of Myzostoma, this differentiation takes place only during maturation of the fertilized ovum. However, in some ova of direct unequal division, the factors determining the equal segmentation are already present from maturation on. In fact, it is known from the observations by Bonnevie (1910) and by F.R. Lillie (1912) that, during the second maturation mitosis, the asymmetry of the cytoplasm of Nereis, which is the cause of unequal division, manifests itself already in a special state of the sperm-aster.

It seems much more legitimate to consider, as the quantitative difference, a discrepancy in concentration or density of certain substances. The polar cytoplasm is a particularly dense cytoplasmic collection which seems to push back the vitellus and which is distinctly delimited from the other regions of the ovum. The presence of this plasm acts as a corollary by its isolation into a lobe at the moment of mitosis of the ovum. We demonstrated above that this substance is especially refractory to the cytodieretic processes and that the mitotic groove is incapable of penetrating into this substance. It could be /190 assumed that, in all ova of unequal segmentation, there exists an analogous substance which, instead of being condensed at the vegetative pole, would be distributed over a much larger territory and in a sufficiently uniform manner not to be distinguishable from the ambient cytoplasm. Just as the condensed substance of the polar lobe causes a deviation of the division groove, the possible substance of ova with direct unequal division could push back the spindle - and thus the groove - to either side of the ovum. If this were so, the division of animal and vegetative fragments of an ovum with polar lobe on the one hand and of an ovum with direct unequal segmentation on the other hand would have to be different. Although merogony of this latter category of ova has never again been realized after the ancient and loosely oriented experiments by Delage (1899) on Lanice, a comparison between the results obtained on ova with polar lobe of Dentalium and of intermediary type ova of Chaetopterus is nevertheless of great interest. In fact, on the one hand the experiments by Wilson (1904) have shown that an amputation of the polar plasm of the virgin ovum of Dentalium* resulted in an equalization of division; on the other hand, Whitaker and

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* In the annelid Sabellaria, Hatt (1932) obtained an ablation of the vegetative pole (about one third of the ovum) during maturation. This animal fragment, however, divided in an unequal manner, with one polar lobe slightly reduced; it is not known at what moment the plasmotic condensation of this species occurs; possibly it is retarded, as is the case in Myzostoma. On the (cont'd)

Morgan (1929) obtained the separation of the vegetative half and the animal half of a virgin ovum of Chaetopterus. These ova were in the metaphase of first maturation; from work done by F.R.Lillie (1906) we know that, starting from this stage, the polar cytoplasm is already differentiated. Conversely, we know that this ovum has only a reduced polar lobe, that its achromatic spindle is eccentric, and that it closely resembles an ovum of direct unequal division. The results by Whitaker and Morgan demonstrate that, if the vegetative fragment divides in the usual manner, the animal fragment - while not forming a polar lobe - divides in an unequal manner; consequently, in an ovum of the type of Chaetopterus, the factors of unequal division are not uniquely localized in the small cytoplasm of the vegetative pole and actually ascend to the animal half /191 of the ovum. This fact is easily reconciled with the hypothesis established by us: The cytoplasm, but in the diffuse state, would occupy an extended portion of the ovum and thus be responsible for the asymmetric structure leading to the recession of the achromatic spindle into an eccentric position.

Previously, in our studies on the Barnea ovum, we conceived a hypothesis of a different type: Based on the fact that ultraviolet radiation, together with equal segmentations, also produced an increase in permeability to salts, we suggested the possible intervention of a cortical permeability gradient for explaining the unequal division. The increase in permeability under the effect of ultraviolet radiation is even more reliably established at present; the permeability to salts had been demonstrated by an entity of highly convergent although only indirect proofs. At present, we have been able to demonstrate by direct means (rate of swelling in hypotonic solution), in the same ovum of Barnea (since ova of Aplysia and Myzostoma are rather unsuitable for this type of experiment) an increase in permeability to water after irradiation with a mercury vapor lamp. Despite the fact that we cannot as yet reliably correlate the unequal segmentation and the permeability, it should be noted that the concept of physiological properties localized in certain portions of the cortex is by no means contradictory to the assumption of an unequal distribution of certain substances at the interior of the ovum. It even seems that the cytoplasms are intimately connected with the cortex; in fact, in Chaetopterus, according to descriptions by Lillie (1906), the polar plasm is nothing else but a cortical differentiation. Conversely, let us mention an interesting observation by Schleip (1914); after centrifuging Clepsine ova, this author was able to observe that the animal and vegetative polar cytoplasms of this Hirudinea shift along the cortex while remaining closely adherent to it.

Resumé /192

1) With the exception of Hirudinea and Oligocheteae, in which a polar cytoplasm exists at the animal pole and another one at the vegetative pole, characteristic cytoplasms in Spiralia are encountered only at the vegetative pole of ova forming a polar lobe from the very first mitoses.

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* (cont'd) other hand, Wilson (1929) obtained merogonies in Chaetopterus by centrifugation at various stages. Since centrifugation has an influence on the mitosis, the results of this author have no bearing on the subject of interest here.

In general, this cytoplasm pre-exists in the virgin ovum. Nevertheless, in Myzostoma, in contrast to certain previous observations, this cytoplasm is built-up during the maturation. The green substance, described by Driesch, is nothing but an inclusion without morphogenetic significance, as proved in centrifugation experiments.

2) The sensitivity of the Myzostoma ovum to agents susceptible of reducing the inequality of divisions is relatively slight, being zero with respect to centrifugation and low with respect to ultraviolet irradiation. Nevertheless, in a certain number of ova, these agents prevent the formation of a polar lobe and produce first signs of a division which has all appearances of becoming equal. However, the groove stops at the level of the polar cytoplasm, after which it recedes again and leaves the ovum unsegmented.

3) Irradiation by ultraviolet rays, which equalizes the direct unequal divisions, results in disappearance of the polar lobe and in a tentative equal segmentation of the Myzostoma ovum. Between the two types of ova, only quantitative differences can be detected. Since chronological differences can be excluded, we believe in the hypothesis of a difference in distribution and condensation of a characteristic substance refractory to the cytodieretic processes, of which only the condensed form results in the constitution of a morphologically definable cytoplasm.

3. Equivalent of Unequal Divisions in the Differentiation without Cleavage of the Chaetopterus Ovum

In Spiralia, segmentation and morphogenesis apparently are intimately correlated. It is known (see Penners, 1921; Tyler, 1930) that the disorders of segmentation have a repercussion on the final fate of the embryo. Nevertheless, ever since the remarkable work done by F.R.Lillie (1902, 1906), we also know /193 that the unsegmented ovum of Chaetopterus is capable of differentiating to the extent of showing, along general lines, a structure recalling that of the trochosphere. These data may seem contradictory. In reality, the observations made by us on these ova, with differentiation but without cleavage, show again the essential morphogenetic significance of mitoses. In fact, each monasteric mitotic cycle is accompanied by cytoplasmic lobulations whose localization is closely related to the final situation of divisions that should have occurred.

During our stay at Naples, we made an attempt to use the Chaetopterus ovum as experimental material. However, most likely the season was too advanced (end of August and September) so that we never arrived at a correct fecundation: A minority of ova segmented normally while others, simply activated by the contact with the sperm, differentiated without dividing.

Their monasteric mitotic cycles (verifications on microsections of fixed material) showed a perfect synchronism with the normal divisions; this same synchronism persisted for the monasteric mitoses of virgin ova activated by addition of a small amount of potassium chloride to the sea water. The two categories of activated ova exhibited no difference whatever.

The first cycle, just as in the normal ovum, starts with a piriform stage

followed by a polar lobe (Fig.15). However, as indicated in the same sketch, this lobe soon becomes eccentric and seems to shift toward one of the sides of the ovum, without showing any trace of division; soon thereafter, the ovum rounds off.

At the moment at which the normal ova start their second division, the polar lobe of the unsegmented ovum reappears, just as in the CD cell of the normal ovum. The evolution of this second lobe is completely similar to that of the first.

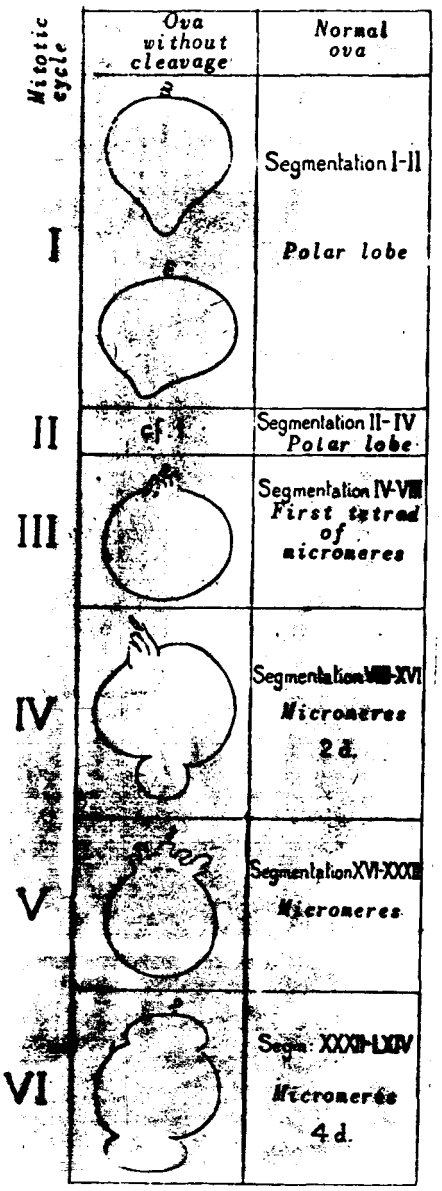

Fig.15 According to Sketches Made in Vivo; Evolution of Ova without Cleavage Compared with Normal Segmentation.

After a new period of rest, the normal ova divide (IV and VIII) to form the first tetrad of micromeres at the animal pole. This time, the undivided ova show no polar lobe and faintly outline only vague tremors at the vegetative pole; however, at the animal pole, a series of lobulations appears, giving this pole a polypiform budding aspect (Fig.15). /194

The next cycle (VIII to XVI), in the normal ovum, is characterized by division of the first tetrad and formation of a second tetrad of micromeres. Among the latter, the one corresponding to the quadrant D, i.e., 2d, is of special importance. Larger than the others, it constitutes the first somatoblast from which the "somatic plate" is derived, forming almost the entire truncal ectoderm. The fourth cycle, in the unsegmented ovum, is characterized by strong lobulations of the animal pole but also by formation of protoplasmic lobes at the vegetative pole (Fig.15). The fifth cycle, in the normal development, corresponds to the division of the first two tetrads of micromeres and to the formation of the third tetrad. The lobulations of the unsegmented ovum take place exclusively at the animal pole (Fig.15). In the sixth cycle, the second somatoblast (4d) is formed. This coarse cell, issuing from the quadrant D, constitutes the source of the entire mesoblast. This important stage manifests itself in the undivided ovum by lobulations of the vegetative pole accompanying those of the animal pole.

The subsequent evolution becomes less /195 clear; the lobulations of the two poles amplify and finally lead to a complete modification of the ovular substances. After several hours, the peculiar phenomena of "intercellular

gastrulation" appear, as described by Lillie; if the evolution progresses sufficiently far, formation of ciliated larvae will take place. In this respect, we need add nothing to the authoritative descriptions by F.R.Lillie.

In summation, the first mitotic cycles constituting the quadrant D as well as the expulsion of the two somatoblasts find their equivalent in the undivided ovum in lobulations of the vegetative pole; the formation and division of the micromeres manifest themselves by movements of the animal pole.

Even if differentiation had taken place without cleavage, it will nevertheless be accompanied by physiological equivalents of unequal divisions. These cytoplasmic manifestations occur at the moment of monasteric mitosis. It is legitimate to believe that they originate from the interaction between nuclear constituents and cytoplasm.

In any event, monasteric mitosis takes place at the center of the ovum; thus one can definitely exclude any intervention of an unequal division of the centrosomes or any other hypothesis that attributes the unequal segmentations and their highly accurate localizations to structures or particular properties of the achromatic spindle. The directive factor of unequal divisions is nothing else, and can be nothing else, than a cytoplasmic factor.

Conclusions

At present, without repeating the details given at the end of each of the three Sections of this paper, we would like to present the picture along general lines.

Primarily, we demonstrated that the unequal segmentations, even those of a less characteristic type (Aplysia or Myzostoma), arise from a common mechanism with purely quantitative variations. A study of the mechanism of these inequalities thus is a problem that can be attacked in its totality and in the same manner for all of the species involved.

With respect to the nature of this mechanism, we were able to discard several factors which might have been in question: neither an unequal division of the centrosome nor a particular asymmetric structure of the achromatic spindle can be considered here. In addition, intervention of dyschronism can be eliminated, from the viewpoint of experimental production of equal segmentations as well as from the viewpoint of differences (purely quantitative, as demonstrated) between the various types of unequal division. /196

Conversely, we were able to formulate a logical and plausible hypothesis, useful as a guideline for later studies: a different distribution and condensation of substances analogous to those of the polar cytoplasms, which latter are substances capable of offering a certain resistance to cytodieretic processes. On the other hand, just like the polar cytoplasms, these substances have the property of germinal localizations, thus offering a reliable explanation for the link between unequal segmentation and morphogenesis.

Specifically, in Aplysia, we were able to observe morphological as well as

physiological changes in the ovum, taking place toward the end of maturation and slightly thereafter. Nevertheless, it was impossible to determine whether these changes had any correlation with the unequal segmentation or with the morphogenesis. Other results also remain somewhat enigmatic: for example, the action of centrifugation and especially its antagonistic effect to irradiation, as observed in Aplysia.

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